FIRST ANNUAL RESEARCH STATUS REPORT

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RESEARCH CONDUCTED UNDER CONTRACT N ONR 222 (28)

BETWEEN THE

REGENTS OF THE UNIVERSITY OF CALIFORNIA

AND THE

OFFICE OF NAVAL RESEARCH

BY THE

NAVAL BIOLOGICAL LABORATORY

UNIVERSITY OF CALIFORNIA

DEPARTMENT OF BACTERIOLOGY

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31 December 1954

From: Ralph S. Muckenfuss, Scientific Director, University of California contract Nonr 222(28) with the Office of Naval Research

To: Armed Services Technical Information Agency, Documents Service Center, Knott Building, Dayton 2, Ohio

Encl: First Annual Research Status Report

1. The Annual Research Status Report for 1954 of contract Nonr 222(28) is herewith submitted. Five copies are enclosed for your office. This research represents the joint effort of civilian scientists employed by the University of California under the above contract, and of military personnel of the Naval Medical Research Unit #1 and Naval Biological Laboratory.

Ralph S. Muckenfuss

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FIRST ANNUAL

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1 July 1954 - 31 December 1954

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NAVAL BIOLOGICAL LABORATORY

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The research program proposed under ONR Contract No. 222(28) emphasizes two basic problems: (1) a study of the possibilities of producing prophylactic agents such as immune sera and vaccines against one of the vesicular viruses of domestic animals, vesicular exanthema of swine (VEV), and (2) a continuing effort to characterize some of the fundamental properties of the viruses of vesicular exanthema and vesicular stomatitis (VS).

Currently the major emphasis is being placed on determining the possibilities of prophylactic agents for VEV; a problem too pressing to await complete investigation of the fundamental properties of these viruses.

The greatest obstacle to the production of prophylactic agents is that the host range of VEV is limited to the definitive host, swine. This immediately imposes restrictions on both the ability to produce and quantitate the virus, and on the opportunity to test experimental prophylactic agents. The first successful cultivation of VEV in tissue culture was reported from this laboratory in 1954 (McClain, Madin and Andriese 1954) thus indicating that such an approach to the problem of production and quantitation of antigenic material was feasible, and that the "bottleneck" of host specificity has been overcome.

This report has a three-fold purpose: (1) to present a complete review of our knowledge of VEV written in cooperation with Dr. Jacob Traum of the United States Department of Agriculture, (2) to present the experimental problems and results associated with tissue culture and (3) the behavior of VEV in this host system.

PART I - VESICULAR EXANTHEMA OF SWINE

Vesicular exanthema is an acute, febrile, infectious viral disease of swine, characterized by the formation of vesicles on one or more parts of the body. The parts most commonly affected are the snout, lips, tongue, oral cavity, sole, interdigital spaces, and the coronary band of the foot. Occasionally the udder and teats of nursing sows become involved. Occult cases are occasionally encountered.

The course of the disease is usually about 1 to 2 weeks, the mortality is less than 5 per cent, and recovery following uncomplicated virus infection is complete. The incubation period in both the natural and experimental disease usually varies from 24 to 72 hours, with extremes ranging from 12 hours to 12 days. All ages as well as all breeds of swine appear to be susceptible.

Vesicular exanthema is of great economic importance since the disease causes serious weight losses in fat hogs, slow gains in feeder stock, deaths in suckling pigs, abortions in pregnant sows, and impaired lactation in nursing sows. In addition this condition is clinically indistinguishable from foot-and-mouth disease and vesicular stomatitis in swine, thus requiring expensive quarantine procedures.

The natural disease has been reported only within the United States.

HISTORY

On 23 April 1932 a disease afflicting only swine and clinically indistinguishable from foot-and-mouth disease was reported on a ranch near Buena Park, Orange County, California. Quarantine and inspection of the entire area was immediately instituted by State and Federal authorities. On 28 April, two additional ranches near the original focus of infection were found to harbor infected swine. Routine inspection on 30 April showed that the disease was also present in Bellflower, Los Angeles County, on two adjoining ranches, some 15 miles distant from the Buena Park foci. By 4 May the disease had spread to a third neighboring ranch and this was the extent of the infection as it appeared in Los Angeles County. The infection was then discovered on a ranch located about 2 miles north of the original Buena Park focus on 3 May, thus ending the spread of infection in Orange County. Inspection of a ranch in San Bernardino County on 5 and 6 May showed the disease to be present although separated by 40 to 50 miles from the other two foci. The San Bernardino County infection was the last to be reported and represents the extent of the 1932 outbreak. The disease was diagnosed as foot-and-mouth disease and all animals directly and indirectly involved in the outbreak were slaughtered and buried, the premises washed with lye solution and all livestock was excluded for 30 days. Indemnities of $203,328 for the loss of the 18,747 swine, 46 cattle and 24 goats were paid jointly by the State of California and the Federal Government (Mohler and Snyder, 1933; Duckworth and White, 1943).

The virus from the 1932 outbreak failed to induce lesions in 24 guinea pigs, 2 calves, 2 heifers, 1 adult cow, and 2 horses (Traum, 1934). On the basis of the above tests the diagnosis of foot-and-mouth disease was made even though Traum (1933a) recognized that it was rather atypical. All virus collected during the outbreak was ordered destroyed.

In March of 1933, a disease again restricted to swine and clinically similar to the 1932 outbreak appeared in San Diego County, California, 100 miles distant from the 1932 foci. The original focus and the immediately adjoining ranch were both found infected on 20 March and the infection was reported from a third ranch on 31 March and at a fourth ranch a few days later (Traum, 1933b). Virus from this outbreak was collected and tested in a variety of animals. Infection was established in all of 15 swine, in 4 of 9 horses but in none of 7 cattle and none of 37 guinea pigs (Traum 1934). Similar results on a larger number of animals were obtained by Mohler (1933a) and Reppin and Pyl (1935). Observers of the animal tests, with experience in foot-and-mouth disease, saw no definite points of clinical difference between that disease in swine and the one produced by the San Diego virus. The above animal tests permitted no official diagnosis although the slaughter and quarantine methods were again practiced. Idemnification in the amount of $45,350 was made for the slaughter of 5,578 animals (Mohler, 1933b; Duckworth and White, 1943).

Cross immunity tests against vesicular stomatitis virus (types Indiana and New Jersey) and foot-and-mouth disease virus (types "A", "O", "C") showed that the San Diego virus was immunologically distinct from both these viruses. In comparing the 1932 and 1933 outbreaks, it was noted that, "The true classification of the virus causing the 1932 swine outbreak of foot-and-mouth like disease must be considered as not having been definitely determined, even though a diagnosis of foot-and-mouth disease had been made and eradication carried out accordingly. It is believed, if more horses had been used in the tests, that lesions would have been produced, thus making the virus of 1932 and 1933 alike in every respect" (Traum 1934). Following the 1933 outbreak a new disease of swine was recognized, in the following statement, "Thus, we are confronted by a vesicular disease in swine, which so far has shown as much difference in experimental inoculations and immunological tests from both vesicular stomatitis and foot-and-mouth disease, as does foot-and-mouth disease from vesicular stomatitis and, although great similarity exists between the viruses of vesicular stomatitis and foot-and-mouth disease, we have been designating them as separate diseases. It therefore seems that with the information at hand the swine disease discussed above should be recognized as a new entity. Vesicular exanthema of swine is suggested as a name for this disease" (Traum 1934).

In June of 1934, 15 months after the San Diego outbreak of 1933, the disease appeared on a garbage feeding hog ranch near San Jose, Californa, some 500 miles distant from the San Diego foci (Duckworth 1953; Duckworth and White, 1943). During the next 3 months the infection spread over 5 counties in Central California involving 27 ranches, and four ranches in Los Angeles and San Bernardino Counties, 400 miles to the South. A total of 31 premises and 95,000 hogs were affected. All of the cases occurred on hog ranches practicing garbage feeding, and as had been the case in the 1932-33 outbreaks, only swine were involved. Virus recovered from this outbreak regularly infected swine. Horses were only mildly susceptible, whereas cattle and guinea pigs were completely refractory (Duckwork and White, 1943).

In the absence of indemnification, the original slaughter program was not employed, but instead a rigid quarantine was imposed on infected premises until all evidence of the disease had disappeared. Trucks used for hauling garbage were disinfected upon departure and steps were taken to insure that truck drivers and ranch attendants did not contact other hog ranches or livestock premises (Duckwork and White, 1943).

In 1935 the disease reappeared on 4 of the premises infected in 1934 and involved about 13,000 hogs. The disease was relatively mild and the quarantine measures were again imposed.

In 1936 the disease struck first on 8 April in San Diego County on one ranch and infected approximately 90 per cent of the animals (Duckworth, 1953). The infection did not spread to neighboring ranches but instead, on 24 April appeared in the San Francisco Bay Area, 500 miles north of San Diego. By 20 June, 13 more or less widely separated premises were involved (Duckworth, 1953).

No cases were reported from 20 June 1936 until 4 December 1939, despite the fact that regular inspection of garbage feeding hog ranches was carried out. Los Angeles County with the largest hog population in the state had been free of the disease for 6 years prior to March 1940 (Hurt, 1940 - 1941).

On 4 December 1939, an outbreak of vesicular exanthema was found on one garbage feeding hog ranch in San Mateo County. An immediate and rigid quarantine was imposed on the infected area. Slaughterers, commission firms, and stockyard officials were ordered not to accept shipments of hogs from the infected area. This economic quarantine was relaxed only when a definite diagnosis had been made, and then only swine coming from non-infected premises could be slaughtered. In addition all hogs going to slaughter from the area were individually examined (Duckworth and White, 1943; Duckworth, 1953).

In spite of all the quarantine efforts, 223,000 hogs, on 123 premises, located in 25 counties became infected. Within 6 months, one-fourth of the state's hog population was involved.

From June to October of 1940 there was a respite from the disease, but on 5 October 1940, the virus reappeared in 12 counties in the Central portion of the state and in December of 1940, appeared in Los Angeles County, involving 57 premises and 54,250 additional swine. During the year 1940, 277,250 swine on 169 premises were infected. The 1940 outbreak was noted for the severity of the disease, and by the fact that 7 of the foci were grain feeding ranches, and one was a stockyard, marking the first time that infections were observed on non-garbage feeding premises (Duckworth, 1953).

After 1940 the recording of individual outbreaks was discontinued, in lieu of which the total number of outbreaks for any one calendar year was substituted. Since 1940 the disease has recurred each year. The number of swine infected have varied from 439,876 head in 1944 to 84,442 head in 1951.

Table 1 modified from Duckworth (1953) shows the number of outbreaks, their place of origin and the number of swine involved per year for the first 20 year period, (1932 to 1952). Figure 1 shows the counties involved in the epizootiology.

In 1948 and again in 1949, the virus appeared in a number of swine being shipped to the port of Honolulu. These animals had been loaded from California ports, and, it is assumed, had come in contact with the virus prior to or during shipment. Prompt quarantine and slaughter before reaching Hawaii prevented the spread of the disease to the Hawaiian mainland. No outbreaks have ever been reported in Hawaii.

On 16 June 1952 vesicular exanthema appeared at a plant, manufacturing biologics in Grand Island, Nebraska. The source of this infection was traced to Cheyenne, Wyoming, where hogs had been fed garbage from transcontinental trains whose point of origin was California. It is assumed that contaminated pork scraps were the source of the virus. Prior to detecting the disease at Grand Island, some of the hogs were shipped to the Omaha stockyards, where they were in turn resold. In this manner the disease immediately fanned out and by 29 July, just 43 days after discovery of the disease in Nebraska, 19 states were placed under Federal quarantine for vesicular exanthema. On 1 August 1952, a state of emergency was declared by the Secretary of Agriculture, thus providing Federal support for an active eradication program including slaughter and payment of indemnities where such were deemed necessary (Simms, 1953).

From June of 1952 to September of 1953 a total of 42 states and the District of Columbia had experienced the disease (Mulhern, 1953). Figure 2 shows the numbers of infected-exposed swine from the period June 1952 to February 1954. The states of California and New Jersey are not included in these totals since the disease has become estabished in the raw garbage feeding areas of these states. Thus from its initial appearance in 1932 and its apparent confinement to the state of California for 20 years, vesicular exanthema is now in a position to menace the swine industry of the entire nation to an extent that can only be assessed with the passage of time.

CLINICAL PICTURE

Vesicular exanthema is clinically indistinguishable in swine from either foot-and-mouth disease or vesicular stomatitis (Traum, 1934; British Report, 1937). The incubation period in both natural and experimental vesicular exanthema usually varies from 24 to 72 hours, with extremes from 12 hours to 12 days (Madin and Traum, 1953).

The introduction of virus into susceptible swine usually produces vesicles on the snout, lips, tongue and mucosae of the oral cavity and on the sole, interdigital spaces and coronary band of the foot. Occasionally lesions may appear on the teats, particularly of nursing sows (Traum, 1936; Hurt, 1940-1941), and on the skin covering the metacarpus and metatarsus (British Report, 1937). Inoculation of the virus intradermally into the snout and or mucosae of the oral cavity by needle or scarification usually produces the classical picture, first the "primary" lesions at the site of inocuation in 12 to 48 hours and then "secondary" lesions elsewhere 48 to 72 hours later. Inoculation of the virus via the subcutaneous, intramuscular or intravenous routes is usually followed by the appearance of vesicles at any of the susceptible sites within 24 to 96 hours after inoculation.

In the typical case a diphasic symptomatology results. In phase 1, lasting from 48 to 72 hours, there is a characteristic rise in temperature (figure 3), and the appearance of primary vesicles which is usually associated with anorexia and listlessness. The primary vesicles consist of blanched, raised areas of epithelium varying from 5 to 30 mm in diameter and raised to 10 to 20 mm in height and filled with a serous fluid rich in virus. Such vesicles

resemble the "blister"formation accompanying burns or excessive dermal friction. Primary vesicles follow along the original paths made by the inoculating needle. The epithelial coverings may "lift" with the slightest pressure revealing a raw, bleeding and exceedingly sensitive corium which is subsequently covered by a yellowish fibrinous membrane (Traum, 1936; Crawford, 1937 and British Report, 1937).

The primary lesions usually spread to involve the adjacent mucosa of the lips and cheeks. This spread is probably derived from virus liberated from the primary vesicles, as new lesions often follow the path taken by fluid escaping from ruptured vesicles. The subcutaneous tissues of the snout and tongue may become hyperemic and swollen, and are sensitive to pressure. As a result the snout may appear bulbous and the swelling of the tongue lead to attacks of slobbering (Hurt, 1940-1941). Phase 1 is almost invariably accompanied by serious temperature changes which occasionally are as high as 108 F (White, 1940) but more commonly between 105 to 106 F. The end of phase 1 is usually signified by a decline in temperature and rupture of the "primary" vesicles.

Phase 2 is ushered in by the formation of "secondary" vesicles on the sole of the foot, between the interdigital spaces, and at the junction of the epithelium and nail of the foot (coronary band). In all probability phase 2 represents the end of the incubation period of the viremia. The initial appearance of foot lesions is usually indicated by a characteristic hesitant gait, described by field veterinarians as "ouchy". The animal may continue to walk in this halting fashion, or may simply refuse to move until the pain and swelling have decreased. In severe attacks an edematous swelling of the legs and joints may be present. Phase 2 usually lasts for 24 to 72 hours following phase 1 and is terminated by the rupture of the secondary vesicles, a subsidence of pain and the gradual resumption of normal living habits. During both phases, 1 and 2, the animal may refuse food and this coupled with the severe pyrexia literally "melts" the weight from market animals.

Recovery of uncomplicated cases is usually prompt and with sequellae. The healing of very severe foot lesions may result in the formation of nodules of granulation tissue which arise from the sole of the foot prior to replacement by the normal epithelium. Pyogenic bacteria may gain entrance through the damaged epithelium and cause severe and even fatal secondary infections. A certain proportion of cases lose the hoofs of the infected feet and replacement may take from 1 to 3 months during which time the animal may be partially lame and is constantly subject to secondary bacterial invaders. It is of interest to note that the junction of the old and new nail is marked by a dark brown or black line, rendering a diagnosis of vesicular exanthema infection probable even though all acute symptoms have disappeared.

In addition to the above symptoms, severe attacks of diarrhea accompanying the infection, an apparent increase in the abortion rate of infected sows and a general drop in milk production in lactating sows were reported by Hurt (1940-1941). Wictor and Coale (1938) and Mott, Patterson, Songer and Hopkins (1953) noted that a mild infection may be missed completely, thus yielding a source of "occult cases".

PATHOLOGIC PICTURE

Lesions directly attributable to the virus, other than vesicle formation have not been described. Histologically the vesicle consists of a circular area, "eaten" out of the stratum malpighii (figures 4 through 7). The center of the area is usually devoid of anything but cellular debris and serous fluid. The first series of cells lining the area usually show cytoplasmic degeneration with pyknotic nuclei and even karyorrhexis. Cells further from the center show a ballooning of the cytoplasm, a marked stretching of the intracellular bridges and considerable intercellular edema, bordering on spongiosis. There may be a few normal epithelial cells surrounding the region of edema, but usually one vesicle tends quickly to blend into another. The subcutaneous connective tissues show acute inflammatory changes characterized by congestion, edema, hemorrhage and polymorphonuclear infiltration. In some cases where the integrity of the basement membrane has been disturbed some of the cellular elements "spill over" into the stratum malpighii (Madin, 1954 a). Inclusion bodies have not been reported.

The pathology described above is very similar to that described by Chow, Hanson and McNutt (1951) for vesicular stomatitis, and by Frenkel (1949) for foot-and-mouth disease.

EXPERIMENTAL HOST RANGE

Vesicular exanthema virus shows a marked predisposition to porcine epithelium and an almost equal indisposition to the tissues of other species.

Traum (1934) was the first to study the host range of the virus when he showed in the original outbreak of 1932 that inoculation of material into guinea pigs, swine and a limited number of cattle and horses produced lesions only in swine. In the 1933 outbreak inoculation of these same species provided consistent "takes" only in swine with mild reaction in 4 of 9 horses. These findings were confirmed by Reppin and Pyl (1935) and Mohler (1933a) and they further indicated that the horse was easier to infect than previously suspected. Crawford (1937) isolated 4 strains of the virus, "A", "B", "C" and "D" and found that while all 4 were infectious for swine, only types "B" and "D" were infectious for the horse. Crawford attempted to passage the virus to sheep, goats, guinea pigs, white rats, white mice and hedgehogs and found that none of the 4 strains produced any visible reaction in these species. The British Workers (1937) working with one strain (unspecified) were able to infect swine, but not horses, cattle, sheep, goats, guinea pigs, rats (Rattus norvegicus) and hedgehogs. Madin and Traum (1953) reported negative results with the chick embryo, rabbit, and several strains of adult and suckling mice including the agouti, C57 black, hybrid black and Namru. Man is not susceptible.

Madin and Traum (1953) reported that the hamster could be infected with the 1940 "A" and "B" strains if the inoculations were made intradermally over the abdomen. Reliable and clear cut vesicles were formed at the site of inoculation within 24 hours and were accompanied by a significant pyrexia. The vesicles ruptured shortly after formation, and no further reactions were visible. Inoculation of hamsters with the current 1948 "A" and 1951 "B" strains gave completely negative results. It is presumed that sufficient differences exist among the

various strains of the virus, as has already been indicated by Crawford (1937) to account for the alternate failures and successes with this particular host. The current status of our knowledge regarding the hamster indicates that it does not represent a reliable small laboratory animal for this virus. In addition to the above Madin (1954 a) has failed to infect the white rat and guinea pig with the 1948 A and the 1951 B strains, although complement fixing antibodies are produced in the guinea pig. The ferret has also been found refractory. Brooksby (1954) has reported negative results with strains 1934 B and 1943 101 in suckling and young adult white mice. Bankowski and Wood (1953) found that dogs were irregularly susceptible to types 1948 A, 1951 B and 1952 C. Intradermal lingual inoculation produced mild lesions at the points of inoculation, characterized by erosion of the epithelium, blanching and extension. The virus was recovered from the spleen of one febrile but not from two afebrile animals. The lack of a reliable laboratory host for this disease means that all work must be done in swine, and explains in part why, after approximately 20 years of work with this virus, so little is known about it.

The limited host range prompted investigations in the field of tissue culture. McClain, Madin and Andriese (1954) reported the first successful cultivation of vesicular exanthema virus. These authors showed that strain 1951 B could be propagated on embryonic swine skin and that cytopathogenic effects were produced. Subsequent to this Madin and McClain (1954) have successfully propagated the virus on monolayer cultures of adult swine kidney and testicle following the general method used by Dulbecco and Vogt (1954) and Youngner (1954), for the propagation of poliomyelitis virus. These initial efforts in this field have made possible expanded research on vesicular exanthema virus, since for the first time, an experimental host other than live swine is available.

ETIOLOGY

Filtration of infectious ground vesicle covering material through gradacol membranes showed that the virus is capable of passing membranes of 44 mμ average pore diameter (APD) but not 39 mμ APD. The size of the virus is calculated to be from 13 to 20 mμ (Madin and Traum, 1953). Brooksby (1954) reported that the 1934 B and the 1943 101 strains passed gradacol membranes of 110 mμ and 70 mμ (APD) but not 37 mμ (APD). The virus has been preserved for as long as two and one-half years at ordinary refrigerator temperatures in the form of unground vesicle coverings stored in 50 per cent glycerine phosphate buffer. It will retain its infectivity for as long as six weeks at room temperature, when diluted 1-10 in 1 per cent ordinary peptone solution, and will survive for at least 24 hours at 37 C in Sorensen's buffer. Storage at -10 C is routinely used (Madin and Traum 1953).

In a series of feeding experiments Mott, Patterson, Songer, and Hopkins (1953) showed that infected meat scraps were infectious after storage at 7 C for 4 weeks and at -70 C for 18 weeks. Traum and White (1941) placed infected vesicle coverings inside the bone marrow cavity of both cured and fresh hams, then refrigerated both overnight. Both hams were then "cooked" at 184 F under 10 lbs steam pressure for 10 minutes in a garbage cooker. When the vesicle material was recovered, ground, and inoculated into test swine, it proved to be highly infectious. In certain cases where viral suspensions

have lost their infectivity, Madin and Traum (1953) found it possible to "reactivate" them by the addition of 1-1000 cysteine monohydrochloride to the virus suspension. The minimum period necessary to "reactivate" was found to be 8 days, and once "reactivated" remained so over the longest period tested, 262 days. Both Madin and Traum (1953) and Mott, Patterson, Songer and Hopkins (1953) found fresh 2 per cent lye solution a practical disinfectant.

The existence of a plurality of virus types was proved by Crawford (1937) through his work with a series of virus collections made in 1933 and 1934. Four immunological types "A", "B," "C," and "D" were found based on cross immunity tests in swine. Two of the types, "B" and "D," were found to be infectious for swine only, while the other two, "A" and "C," were infectious for both horses and swine. Some differences in the severity of clinical symptoms were noted, for example, both the "B" and "D" types caused more severe reactions than either the "A" or "C." In 1940-42, three immunologically distinct types were recovered in California and were subsequently lost. Contrary to the report however by Madin and Traum (1953) to the effect that all of the types prior to 1948 were lost, two are still available, the 1934 B of Crawford and the 1943, 101 strain collected by Traum (Brooksby, 1954). In December of 1948 Madin and Traum (1953) isolated the 1948 A, in 1952 Bankowski reported the isolation of the 1951 B and again in 1952 the 1952 C and in 1953 the 1953 D (Bankowski et al. 1952, 1953, 1954). Brooksby (1954) has recently compared the first five of these strains and has found them to be distinct antigenic types.

Complement fixation and serum neutralization tests corroborate the immunological identity of the types. Bankowski, Wichmann and Kummer (1953) have demonstrated that the types can be separated by complement fixation even though they did encounter some cross-reactivity. Brooksby (1954) using their method has confirmed these results. McClain, Madin and Andriese (1954) using a different complement fixation technique were also able to separate the "A" and "B" types. Specific serum neutralization, as observed by the failure of the virus to produce cytopathogenic effects in tissue culture in the presence of homologous serum, can also be used to differentiate the types (McClain, Madin and Andriese (1954).

Vesicular exanthema virus produces a viremia which apparently accounts for the formation of the "secondary vesicles". Thus, the virus may be recovered from the blood prior to 72 hours, while the spleen is positive up to 48 hours (Madin and Traum (1953). In a larger series of experiments Mott, Patterson, Songer and Hopkins (1953) slaughtered a group of inoculated swine approximately 6 hours prior to the development of the vesicles (30 hours after inoculation). Feeding experiments in swine using feet and snout, spleen, crushed bone, whole blood, lymph glands, viscera and muscle, resulted in the production of clinical vesicular exanthema. Animals which had been fed feces and urine failed to develop a clinical infection. In the feces-fed group however both test swine were immune to subsequent rechallenge, thus indicating that sufficient virus had been present to immunize these animals. It would appear that the virus quickly becomes widespread throughout the hog's body. Mott, Patterson, Songer and Hopkins (1953) reported that the time of lesion development varied with the tissue fed, and concluded that this time variation was related to the amount of virus available to the test animal. For example, the group fed feet and snout material developed lesions in 40 hours, those fed spleen or crushed bone in 72 hours, whole blood or lymph glands in 96 hours, viscera or muscle only after 6 days.

The ID_{50} of fresh vesicle covering material has been shown to be 1 x $10^{-5.3}$ (Mott, Patterson, Songer and Hopkins, 1953) which is in close agreement with the figure of 1 x 10^{-6} suggested by Madin and Traum (1953). Comparative titrations by various methods of exposure with infected vesicle covering material or infected defibrinated blood indicated that it takes 10 to 100 intradermal-snout MID's to make one intravenous or subcutaneous MID and 100 to 1000 intradermal snout MID's to make one MID via the oral route, (Mott, Patterson, Songer and Hopkins (1953). These same authors noted that when a susceptible animal is exposed to small quantities of virus an occult case of the disease, with subsequent immunity frequently develops.

DIAGNOSIS

The diagnosis of a vesicular disease is not difficult, since the clinical signs of pyrexia, vesiculation and lameness are almost invariably present. The similarity of the clinical syndrone produced by vesicular exanthema, vesicular stomatitis, and foot-and-mouth disease makes the differential diagnosis of a vesicular disease difficult. This clinical similarity is further complicated when the outbreak occurs in swine, since this animal is susceptible to all three viruses.

The present method of differentiating among vesicular exanthema, vesicular stomatitis, and foot-and-mouth disease depends on the differential susceptibility of various test animals. This system is illustrated by table 2 modified from Madin and Traum (1953). Similar schemes have been advanced by Traum (1934) Crawford (1937) and Bankowski (1954), but essentially all are the same and involve the inoculation of different routes of one or more cattle, horses, guinea pigs, and known susceptible swine with virus obtained from the outbreak in question.

The weakness of this system has been pointed out by Madin and Traum (1953), "This system of animal inoculation is satisfactory as long as live virus is available, speed is not critical, typing of the individual virus is not required, and a new vesicular disease has not arisen". This weakness was clearly illustrated in the initial outbreaks of vesicular exanthema in 1932-33, when the investigators found such a system of diagnosis inadequate for reaching a clear cut decision. Because of the drawbacks to the animal inoculation system the investigation of serological methods has received attention. In 1953 Bankowski, Wichmann and Kummer (1953) announced the development of a complement fixation test capable of identifying and differentiating the antigenic types of vesicular exanthema virus. This test employes as antigen, vesicle-covering material obtained from an outbreak, hyperimmune swine serum, and guinea pig complement. The rest of the reagents are standard. It was found that a certain degree of "cross reactivity" among types required that each serum be titrated with homologous antigen to determine the maximum amount of hyperimmune serum which specifically reacted with the homologous virus in the absence of cross fixation with any of the other types of vesicular exanthema virus. In addition the high "procomplementary" activity of swine serum was controlled by the titration of complement in the presence of normal swine serum and each antigen employed in the test. Brooksby (1954) has modified this technique by the addition of sodium polyanetholesulphonate to the swine complement to destroy the third component, (C_3) and thus destroy the complement enhancing effect of swine

serum. These initial attempts at complement fixation have seen preliminary trials in the field, particularly the test of Bankowski, Wichmann and Kummer (1953) and have proven of considerable diagnostic aid in the identification of vesicular exanthema virus types (Bankowski, 1954).

Serum neutralization tests have been briefly described by McClain, Madin and Andriese (1954) using tissue culture of embryonic swine skin as a test host, but such a system is as yet in its earliest developmental stage. Hemagglutination has been unsuccessfully employed by Madin and Traum (1953).

TREATMENT

There is no known treatment for this disease. Certain precautions of a palliative nature may be taken, which will tend to reduce the economic losses from this infection. Weight losses can be reduced if infected animals are placed on soft foods or slops entirely, if they are taken off concrete or similar hard surfaces, and if adequate amounts of clean water are kept before them at all times. Clinically ill animals should be kept under shaded conditions, as the pyrexia coupled with the extreme reluctance to move, makes them susceptible to blistering and sunstroke.

Where infected animals must be maintained in crowded quarters such as during rail shipment, in feed lots, or in slaughter houses, secondary bacterial complications may be markedly reduced by the judicious administration of penicillin and streptomycin.

EPIDEMIOLOGY

Vesicular exanthema is known to be spread by at least two methods, direct contact and the feeding of raw garbage. These two routes of infection can account for the vast majority of the outbreaks, but do not clearly do so for the initial outbreak in 1932 and 1933, and the subsequent epizootics in 1934 and 1939.

Direct contact includes for purposes of this discussion contact with contaminated feed, water and fomites as well as contact with infected animals within the hog's particular environment. It should be pointed out that as a group swine live in most intimate contact, and the exchange of disease agents by either immediate or mediate contact occurs constantly. This may be the reason that vesicular exanthema shows no particular seasonal incidence, inasmuch as the environment suitable to it is reasonably constant.

The work of Mott, Patterson, Songer and Hopkins (1953) is of particular interest in the matter of direct and indirect contact infection. In their experiments a series of susceptible swine were brought into direct contact with donor animals which had been inoculated at 12, 24, 36, 48, 72, 96, 120, 144, 192, 240 and 288 hours previously. They found that the susceptible or recipient animals contracted the disease from pigs inoculated through 120 hours but not after that time. It was reasoned that such donor animals ceased the excretion of virus at about 120 hours after inoculation. To prove this assumption two donor animals were placed in contact with two normal swine in a clean pen for 12 hours. After 12 hours the two donors were withdrawn and placed in a pen with two other recipients. The process was repeated at 24, 36, 72, 96, 144 and 192 hours after inoculation of the donor animals. In each pen

one of the recipient animals was scarified on the snout and feet prior to the introduction of the infected swine. The donor animals showed clinical vesicular exanthema 48 hours after inoculation. The results showed that the recipient animals were positive in the 24, 36, 48, 72 and 96 hour trials, but not in the 12, 144 and 192 hour groups. This data indicated that prior to 24 hours virus was not eliminated by the donor animals, but began shortly after and continued until 96 to 144 hours after inoculation. To determine the extent of environmental exposure possible, two normal contacts were placed in each of 8 infected pens at 0, 24, 48, 72, 96, 120, 144 and 168 hours after removal of the infected swine. In the 72 hour group one of the normal contacts developed lesions. Subsequently it was shown by challenge with live virus that both animals in the 72 hour group had been exposed to the virus, and one in the zero hour group. This erratic pattern of indirect exposure was similar to that found by Crawford (1937).

From the earliest outbreaks until the present it has been noted that the percentage of hogs infected on any given premises or within any given group varies considerably with the outbreak in question (Hurt 1940-41; Duckworth 1953). In some cases only a small percentage or only certain pens or lots would be involved, where as in others nearly 100 per cent would be involved. The reason for such variation had previously been given as a reflection of the virulence of the virus. The experimental data of Mott, Patterson, Songer and Hopkins (1953) concerning the infectiousness in time of the donor animal indicate that it may be the dominant factor in spread by direct contact.

What then is the role of raw garbage as a vehicle of spread? Duckworth (1953) has accused this vector in the following words, "Raw garbage is the source of vesicular exanthema". By this he meant that the evidence gathered over a 20 year period and shown in table 1 indicated that the feeding of raw garbage was the principle vector in the spread and continuation of this disease. Mulhern (1953) has reported that almost all of the outbreaks occurring after the 1952 "escape" of the virus from the confines of California have either had direct or indirect connection with garbage feeding establishments. The link between raw garbage and the virus is supposedly infected pork scraps which act as a reservoir of the disease, (Duckworth and White 1943). This hypothesis gains theoretical support from the feeding experiments conducted by Mott, Patterson, Songer and Hopkins (1953) and from the studies on the survival of the virus by Traum and White (1941) described earlier in this review. There appears to be no reason to assume from these experiments that the virus could not survive in an infected carcass and eventually find its way back to susceptible swine through raw garbage. Whereas this mode of spread explains many of the outbreaks, it does not necessarily explain all of them, for example, the 1932 outbreak. In this case no disease such as vesicular exanthema, excepting foot-and-mouth disease, had ever been reported as occurring in swine. It is particularly significant to recall that California had experienced foot-and-mouth disease in 1924-25 and again in 1929 and that all regulatory officials were peculiarly attuned to "a vesicular disease outbreak". We can be reasonably certain that the 1932 outbreak was the first to occur and had its origin in one of the areas described earlier in this review. From the evidence available at that time and from a subsequent review of this evidence the outbreak in two of the areas, Orange and Los Angeles Counties, was not related to the outbreak in San Bernardino County. Thus two separate foci were apparently present almost

simultaneously. The ranches in Orange and Los Angeles Counties, obtained their garbage only from domestic sources by contract. The San Bernardino County premises could possibly have purchased garbage from a foreign ship through a contract with the City of Long Beach, but it is highly doubtful that any significant amount of such garbage found its way to the hog ranches. In this respect it is important to note that since the 1929 outbreak of foot-and-mouth disease, a regulation had been in effect that all ships were forbidden to bring garbage into ports.

One year later the second outbreak occurred, this time 100 miles south of the 1932 occurrence but again on a garbage feeding hog ranch. Was there a link between the 1932-33 outbreaks? The only association outside of raw garbage was the fact that one of the ranches involved in both the 1932-33 outbreaks was owned by the same family. There is no evidence however that man had been instrumental in transmitting the disease in 1933. As near as could be ascertained the 1933 outbreak was a distinct and separate outbreak, similar in many respects to the 1932 occurrence. In 1934 the third outbreak occurred, again on a garbage feeding hog ranch 500 miles distant from the 1932-33 foci. In discussing the 1934 outbreak, Duckworth (1953) pointed out that, "It is inconceivable that infective material of any kind could have carried over from either of the two earlier outbreaks and found its way into a swine herd 500 miles distant 15 to 26 months later". There is also the fact to be remembered that all of the animals in the 1932-33 epidemic were slaughtered and buried and therefore none of these carcasses found their way into the normal trade channels. Thus it would appear that the 1934 epidemic represented yet another separate and distinct focus.

One of the most curious facets of this disease occurred between 20 June 1936 and 4 December 1939 when the frank infection disappeared. Surely, if raw garbage were the only mode of transmission there were enough swine products theoretically containing virus in the normal trade channels, since from 1934 to 1936 a total of 127,000 infected animals had gone to the slaughter houses in the state. Certainly the practice of feeding raw garbage did not disappear during this interval. Why then did no outbreaks occur? It would appear possible that neither direct contact nor raw garbage constitutes the whole story as to the spread and maintenance of this disease. The solution of the epidemiological question propounded by vesicular exanthema virus may ultimately be found along the line suggested by Shope (1954) in that vesicular exanthema is primarily a disease of some "wild" animal and that the domestic swine just happens to be mutually susceptible. Hog ranches which feed raw garbage may serve as a food source for such a wild reservoir and in the course of these events swine are brought into suitable contact with the infection. Such a theory could explain the appearance of the disease at widely scattered points, but it leaves one to ponder why it had not occurred before 1932 inasmuch as the swine practices current then had been in vogue for many years. At present all that can be concluded is that our epidemiological knowledge concerning this disease is too scant to permit a ready answer to the questions of the origin of this virus, and its complete mode of spread. We can acknowledge however that vesicular exanthema represents one of the most intriguing epidemiological problems in veterinary medicine.

CONTROL

Methods for adequately controlling this disease have yet to be found, based on the experience in California. Whether such a statement is applicable to efforts to control the disease on a national scale cannot be determined at this time.

Two methods of control have been used in California: eradication and quarantine. In 1932 and 1933, the time honored methods of slaughter and thorough clean-up so successfully employed against foot-and-mouth disease in this country were applied (Traum 1934), and the disease reappeared in 1934, 400 to 500 miles distant from the first two foci. In 1934 slaughter measures were abandoned and a quarantine of infected ranches was imposed instead (Duckworth and White 1943). The quarantine consisted of embargoes against moving swine from infected premises until all signs of the disease had disappeared. In addition, the movements of vehicles and men were controlled to minimize the possibility of spread by this route. After quarantine had been imposed a differential diagnosis between foot-and-mouth disease, vesicular stomatitis, and vesicular exanthema was made. In stockyards under quarantine affected hogs are released for slaughter in accordance with the meat inspection regulations in terms of the differential diagnosis. Duckworth (1953) has questioned the value of restrictive quarantine, slaughter and disinfection in California and concluded that these methods of eradication were not likely to succeed unless the disease was attacked at its source. He felt that the California quarantine, which at times was quite rigid failed to control the spread of the infection.

In place of the rigid quarantine which has failed to halt the disease in California Duckworth (1953) Mulhern (1953) Shope, Sussman and Hendershot (1952) have recommended the cessation of feeding raw garbage to swine. It is generally agreed that measures less restrictive than this will fail to control the disease at either the local or national level.

The prevalence of the practice of feeding raw garbage to swine is very difficult to assess since no reliable figures are available on a nationwide basis (Haldman, Steele and VanDerweker, 1953). In 1939 a survey of all cities with populations over 10,000 was conducted by Wright (1943). Replies were recieved from 764 or 79.3 per cent of 964 such cities. The replies indicated that a total of 296 cities disposed of their garbage entirely by feeding it to swine while an additional 107 cities disposed of part of their garbage in this manner. Thus, a total of 403 or 52.7 per cent of the 764 cities replying disposed of municipal garbage in part by feeding it to swine. In Maryland, Heyl (1949) found 17 out of 88 communities (20 per cent) fed raw garbage to hogs. Helper (1947) reported that 66 of 168 communities in Michigan or 33 per cent were using this method to some degree. Snyder (1949) conducted a survey of 153 cities having a population of 10,000 or over and found only 19 (12 per cent) using hog feeding as a method of garbage disposal. Rawn (1950) estimated that 31 per cent of all cities in the United States with populations in excess of 5,000 disposed of garbage wholly or in part by feeding it to hogs.

Estimates as to the total number of hogs fed on raw garbage vary but probably do not exceed 1,000,000 or 1.5 per cent of the total hog population. Unfortunately, this practice is one that is concentrated along the North Atlantic seaboard states and California. In the latter area approximately 40 per cent of all slughter hogs raised in the state are fed garbage (Sullivan, Maharg and Hughes 1950). This concentration would permit potential establishment of the disease on a more or less permanent basis and continually menaces the remainder of the hog population with a reservoir of the infection.

The control of raw garbage as a disease vector requires legislative action in the various states. This is well illustrated by the events following the outbreak of vesicular exanthema in 1952 wherein numerous state legislatures began the preparation of bills requiring the cooking of garbage prior to its use as hog food. Table 3 shows the status of such legislation in each of the states, and it indicates that 33 states have now passed legislation regulating raw garbage, 6 have existing regulations, 5 have defeated such measures, and 3 have taken no action. How effectively such legislation will be enforced in controlling garbage borne diseases, and vesicular exanthema in particular, now remains to be seen. It is rather interesting that the two states which have perhaps suffered most from vesicular exanthema, California and New Jersey, defeated outright legislation.

In addition to adequate control of raw garbage, two other control measures remain to be developed. Passive immunization by means of an immune serum has been described by Madin (1954 b and c) and appears to be effective against two of the antigenic types for from 2 to 3 weeks. Such a product should aid in preventing "breaks" during shipment of animals. It should also reduce the amount of infected pork getting back into raw garbage inasmuch as "clean" hogs could then be assured of reaching the slaughterhouse floor free from the disease. The serum should also be of benefit to the farmer in reducing expensive losses in baby pigs, pregnant sows and feeder stock.

The third procedure is active immunization against the infection. Madin and Traum (1953) reported that preliminary trials with a formalinized vaccine made from infected epithelial coverings protected swine for at least six months against direct intradermal challenge of the homologous strain. They also remarked that such a vaccine would not be commercially feasible until a method of producing antigenic material in quantity was available. The report by McClain, Madin and Andriese (1954) of the successful cultivation of the virus in tissue culture indicates that a vaccine may ultimately be added to the armamentarium of the regulatory official. The prophylactic use of immune sera and vaccines would have to take into account the existance of the plurality of antigenic types. While this complicates the problem of prophylaxsis it does not constitute an insurmountable obstacle. Thus, in the not too distant future a combination of intelligent and enforcible legislation against the feeding of raw garbage, accompanied by the judicious use of prophylactic agents when available may ultimately make vesicular exanthema a manageable disease.

PART II. THE IN VITRO CULTIVATION OF ADULT SWINE TISSUE

The announcement by McClain, Madin and Andriese (1954) that VEV could be cultivated in embryonic swine tissue drew attention to the importance of tissue culture in relation to this virus. The original method of cultivation of this virus by McClain, et al. (1954), while suitable for certain research purposes was not readily adaptable to the large scale production of antigenic materials. To overcome this problem attempts were made to produce monolayer tissue cultures as suggested by the work of Dulbecco and Vogt (1954) and Youngner (1954). The advantages of the monolayer tissue culture system were many and varied in theory at least. The rapid dispersal and separation of the desired cellular components from the connective tissue elements by enzymatic activity indicated that much less hand labor would be involved compared to the plasma clot type of culture. The ability to use adult tissue rather than embryonic was a distinct advantage, particularly from the standpoint of availability. If cytopathogenic changes were produced similar to those seen in the embryonic skin then this valuable feature of visable assay could be preserved, and lastly the monolayer system gave promise of being a sound production system for antigenic material for future vaccine studies on relatively homogenous cell preparations.

The majority of published work concerned the use of either monkey or chick embryo tissues, thus necessitating the development of a system for swine tissues.

In a previous report Madin (1954 d) described the salient features of the method modified for the use of swine testicle and kidney cells and the early results achieved with that method. Subsequently a number of changes have been made in the method thus modifying it considerably from the original. For this reason a complete description of the current method is included.

MATERIALS

Sera: Swine serum (SS), ox serum (OS) and lamb serum (LS) are obtained from a local slaughterhouse. The blood of freshly killed animals is collected in large flasks (7 to 10 liters). These flasks were stored at refrigerator temperature until the serum separated, after which it was poured off and seitz filtered. Five hundred units per ml penicillin and 0.5 mg per ml streptomycin were added at the time of bottling and each batch was sterility tested in thioglycollate medium.

Phosphate buffer solution (PBS): Buffer of pH 7.5 was prepared according to the formulae of Dulbecco (1954).

Trypsin solution: The trypsin solution was prepared from Bacto Trypsin 1:250 in a concentration of 0.25 per cent by weight using PBS as a diluent.

Nutrient fluids: The nutrient fluids were of two types: (1) a solution consisting of commerically prepared M-199* solution plus the addition of 10 per cent swine, ox or lamb serum and, (2) a solution consisting of so called CW medium plus 10 per cent swine, ox or lamb serum. The CW medium is one originally

* M-199 solution prepared by Difco Laboratories following the formulae of Morgan, Morton and Parker (1950).

designed by Dr. C. Weymouth and is as yet unpublished, the formulae currently in use being that supplied by Dr. M. Vogt. It consists of the following:

CW media

1. Solution 1

Salt solution with dextrose

Add in order:	Grams per 2 liters
NaCl	14.0
KCl	0.4
$Ca(NO_3)2\ \ 4H_2O$	0.52
$MgCl_2\ \ 6H_2O$	0.20
Na_2HPO_4	0.46
KH_2PO_4	0.20
$NaHCO_3$	4.48
Dextrose	9.60

2. Solution 2

Hypoxanthine	0.050
Glutamine	0.100

*3. Solution 3

l - Lysine	0.160
l - Methionine	0.050
l - Threonine	0.075
l - Valine	0.100
l - Arginine HCl	0.075
l - Histidine HCl	0.075
l - Proline	0.050
Glycine	0.200

* Amino acid solutions should be warmed to aid solution.

	Grams per 2 liters
l - Isoleucine	0.050
Phenyl-l-alanine	0.050
l - Leucine	0.050
l - Tryptophane	0.040
l - Glutamic acid	0.150
l - Aspartic acid	0.060
l - Tyrosine	0.080
l - Cystine	0.015
Phenol red	0.025

4. Vitamins

Thiamine HCl	0.010
Riboflavin	0.001
Pyradoxin	0.001
**Folic acid	0.0002
**Biotin	0.0002

5. Add antibiotics (penicillin, 1000 units per ml; streptomycin sulfate, 0.5 mg per ml)

6. Complete 2 liters

7. Flush with CO_2 to bring to proper pH

8. Sterilize with Selas filters.

In those instances, where the media has stood for considerable periods of time, fresh penicillin is added just prior to use.

Glassware. All glassware is washed as follows:

Glassware is soaked in 0.1 per cent Duponal C solution for a minimum of 1 hour and then cleansed by vigorous brushing. It is rinsed and boiled for 1 hour in tap water, rinsed 3 times in distilled water, and 3 times in deionized water. Sterilization is by hot air oven.

** Unstable, make in larger quantities, a dilution of 1:1000

It is important to note that one of the most important features of clean glassware is the manner in which the used glassware is handled prior to washing. In this laboratory all glassware is discarded in deep pans containing 0.1 per cent Duponal C solution, or where this is not feasible, such as with erlenmeyer flasks, they are individually filled with the solution. Great care is taken to insure that no air is trapped in submerged glassware which tends to leave rings of dried material, which may ultimately be difficult to wash clean. Where infectious material is present such pans containing the glassware in the Duponal C solution are autoclaved and it has been found that this acts as a partial cleansing.

METHODS

Adult swine kidneys. Kidneys are removed from apparently normal 3 to 6 month old swine at the slaughterhouse with the capsule intact and transported to the laboratory as quickly as possible. The capsule is seared and removed with the aid of sterile forceps and scissors. A portion of the cortical area is removed (10-25 grams) and minced with the aid of 2 Bard Parker #11 scalpel blades into 2 to 3 mm pieces. The minced cortical tissue is then washed in PBS to remove excess blood.

To 25 grams of tissue in a 250 ml erlenmeyer flask is added 50 ml of trypsin heated to 40 C. A single extraction process is carried out by stirring the kidney-trypsin mixture on a magnetic stirrer for 10 minutes at 100 rpm. The cell suspension thus extracted from the minced kidney tissue is centrifuged for 2 minutes at 600 to 1000 rpm. The supernatent fluid is discarded, the packed cells are washed once in PBS and recentrifuged. The packed cells are then resuspended in 10 ml of nutrient fluid and are maintained at ice bath temperature. Fresh trypsin solution is again added to the tissue fragments remaining in the erlenmeyer flask, the extraction process repeated, and the tubule suspension collected as noted above until the desired volume of cells has been obtained or until the tissue is exhausted. In each case the first 3 extractions are discarded entirely, and only the packed cells from succeeding extractions are collected in the nutrient fluid. When sufficient tubules have been collected they are recentrifuged for 2 minutes at 600 to 1000 rpm. The packed cells are then resuspended in nutrient fluid such that a 1-10 dilution is obtained and thoroughly but gently mixed with a pipette until evenly dispersed. Additional nutrient fluid is then added to make a dilution of 1-200.

The cells are dispensed with the aid of a Cornwall syringe. As an aid to maintaining a homogenous cell suspension the flask containing the cells is mounted on a magnetic stirrer which causes the fluid to gently rotate, thus preventing settling or clumping during dispensing. A total of 2 ml of culture suspension is used for a standard culture tube (150 mm x 15 mm), 10 ml for a standard bottle (milk dilution bottle) and 70 ml for a Blake bottle. Black rubber stoppers are used for closure. Incubation is carried out without motion at 37 to 39 C, the bottles being laid flat in the trays, while the tubes are inclined at a slight angle. Cultures are usually left undisturbed for 48 hours, after which time they may be examined macroscopically for changes in pH, and microscopically under low power (100x) for evidence of cell growth. The old nutrient is discarded and replaced by fresh after 48 hours and again as needed or at about 5 days. When adequate cell growth has occurred (7 to 12 days) the individual cultures are ready for use.

Adult swine testicle. Testicles are obtained from young adult animals at slaughter and processed in a manner similar to that described for the kidney tissue. The following exceptions are noted. Usually all of the extractions are kept if the testicle tissue looks "good", and the number of extractions needed is less than for the kidney. The old nutrient fluids are discarded after 24 to 48 hours of incubation and fresh fluids added. The fluid is again changed just prior to use usually at 72 to 96 hours after planting.

RESULTS AND DISCUSSION

In previous report Madin (1954d) had indicated that the growth of swine kidney was satisfactory about 60 per cent of the time and swine testicle 75 per cent. It was noted then that attempts were being made to individually differentiate those organs (kidney or testicle) which were most likely to produce a satisfactory tissue culture. It is believed that certain physical characteristics of the individual kidney and/or testicle provide a clue to its tissue culture potentialities. The most striking of these in regard to the kidney is the condition of the parenchyma of the organ. Those kidneys whose parenchyma is friable and loose and in no way fibrous or dense make the best prospects, since in the presence of trypsin the friable tissues separate easily, while with the more fibrous or dense tissues the epithelial cells tend to rupture.

In the case of the testicle a number of closely related physical characteristics appear to have bearing on the growth possibilities. The most striking characteristic is the color and certain accessory features which appear to accompany a particular color. It has been noted that testicles from very young animals have a yellowish hue, and that the cells extracted from such testicles are devoid of oil droplets located within the cells. The testicle from older animals are definitely brown in color, and considerably more fibrous in consistency. The cut surface oozes a slightly viscous cream colored fluid containing enormous numbers of sperm cells. The cells extracted from these are also devoid of oil droplets in their cytoplasm. Between these two extremes is a group of testicles whose color is a reddish brown, the extracted cells of which contain numerous oil droplets within the cytoplasm and very few or no adult spermatazoa. These latter testicles are the only ones which yield cells which produce consistently good growth. Currently, by selecting either kidneys or testicles on the above basis the percentage producing good growth has risen to about 90 per cent for both the kidney and testicle.

It may be of interest to note that the majority of both kidney and testicle cultures are mixed cell types. The kidney cultures are composed mostly of tubular epithelium with a few scattered fibroblasts. These latter cells are usually more prominent in the first few days of the culture and tend to be "crowded out" by the growth of the tubular epithelium so that by the time the growth is ready for virus inoculation a confluent growth of tubular epithelium is available. The testicle culture appears to consist principally of sertoli or interstitial cells and a few fibroblastic elements. In contrast to the kidney however, the longer the culture is held the more fibroblastic elements begin to appear so that the culture soon loses its characteristic testicular appearance. Photomicrographs of the cellular elements with detailed descriptions will be supplied in a subsequent report.

The problem of a completely adequate nutrient medium is still unsolved. The use of the commercially available 199 medium still requires the addition of 10 per cent serum, is expensive, and requires frequent changes, although satisfactory growth is usually obtained. The use of the so-called CW media with 10 per cent serum has so far been satisfactory with both kidney and testicle cells, and possesses the advantage of simplicity coupled with considerable economy. Currently both are being used and equally good growth is being obtained.

The use of sera other than homologous has been attempted and with considerable success. Comparison of swine, ox and lamb serum on the growth of either testicle or kidney tissues indicates that lamb serum produces the most exuberant growth in the shortest time, followed by ox and swine serum in that order. These tests while empirical have been so convincing that 10 per cent lamb serum is now used routinely with both nutrient media.

The problems of growth in a wide variety of containers has as yet not been adequately solved. Growth is adequate and in most instances luxuriant on Blake and milk dilution bottles and in test tubes in from 7 to 12 days with the kidney, and from 24 to 72 hours with the testicle. In petri plates, however, growth is still unreliable on any scale. The problem is not so much the actual lack of growth of cells as it is their inability to cover the plate with any degree of consistency. The reason for this is unknown.

Attempts have been made to cultivate other tissues both of swine and other domestic animals. Adult swine lung, spleen and liver have been grown using the routine techniques. Ox kidney and testicle and lamb kidney and testicle have also been grown and appear to present no difficulties. In addition a wide variety of embryonic tissues have been cultivated.

At present the status of the production of adult swine kidney and testicle tissue culture material per se is sufficiently advanced and on a reproducible enough basis to warrant its use as a routine laboratory tool. Certain aspects of the tissue culture work such as the growth of monolayer films on petri plates are not controlled as yet but this together with the search for improved methods and media are being continued.

Part III THE BEHAVIOR OF VESICULAR EXANTHEMA VIRUS IN TISSUE CULTURE

The fundamental objectives from the applied viewpoint wherein the propagation of VEV in tissue culture is important is the production of antigenic material on a scale sufficient for vaccine purposes. In addition the ability to titrate such antigenic material for infectivity is equally important and the preliminary studies on the behavior of VEV in tissue culture have been directed toward these two objectives.

The salient characteristics of the cytopathogenic changes produced by VEV in tissue culture have been described by McClain et al. (1954) and Madin (1954d). These consist of a rounding of the cytoplasm of the tissue culture cells which eventually leads to death of the cell and its lysis from the glass surface.

The knowledge that VEV could be cultivated in vitro then focussed attention on the need for a type of tissue culture which would meet as many of the following criteria as possible:

It should support the growth of the virus with a sufficiently high titer to be of practical value in the production of antigenic material.

The tissue source for the cultures should be readily available and if obtained from a slaughterhouse it should be a tissue of low economic worth on the consumer market.

The tissue should be from adult animals rather than from embryos. The availability of embryonic material is variable depending on the season of the year and in the case of packing houses directly dependent on the volume of animals being slaughtered. In addition embryos require considerably more hand labor to process. Embryos possess the distinct advantage, however, of being easier to propagate.

The tissue should preferably be an encapsulated organ to aid in minimizing contamination. The cells should be readily available from the organ and should have a long survival time. Once extracted and "planted" the cells should grow to a usable condition in a reasonable length of time.

Aside from the matter of titer which is discussed in detail later in this paper, both of the tissues chosen appear to fulfill the criteria mentioned.

This section of the report describes the work with the first 10 passages of 3 strains of VEV in both adult swine testicle and kidney.

MATERIALS AND METHODS

Propagation of VEV in swine testicle.

Virus. The A strain of virus was derived from a pool of "A" type swine virus dated 2-X-53. The B strain was derived from the 6th swine embryonic tissue culture passage. The C strain was hog virus from a pool of C strain dated 5 August 1954.

Tissue cultures. The testicle cultures were either Blake or milk dilution bottle cultures with luxuriant growth.

Passage method. The original seed virus was diluted 1-10 in the nutrient fluid, and 0.1 ml of this diluted material was inoculated into series of milk dilution bottles containing tissue culture and 9.9 ml of nutrient fluid. This dilution of a 0.1 ml quantity to each 9.9 ml of nutrient fluid in the bottle was consistent throughout, with the exception of C strain virus wherein undiluted seed virus was used to initiate the first tissue culture series; thus making the first passage a 1-10 dilution instead of a 1-100.

Following inoculation each passage was allowed to incubate at 37 C until clear cut cytopathogenic changes were present. The entire virus yield was harvested only when the cell population had been destroyed and released from the glass surface, usually 24 to 40 hours post inoculation. Each passage was distributed in 10 ml vaccine bottles and stored at -17 F.

It is of interest to note that while the first 2 passages on testicle took approximately 40 hours for total cellular destruction, the next 8 averaged 24 hours and in some cases changes could be seen as early as 10 hours post inoculation.

The tenth passages of strains "A" and "B" represented a dilution of the original virus containing material of 1×10^{-19}. The "C" strain of virus represented a dilution of a single exponent less or 1×10^{-18}.

The propagation of VEV in swine kidney.

Virus. The "A", "B" and "C" strains were derived from swine virus dated 2-X-53, 13-VII-54 and 5-VIII-54, respectively.

Tissue cultures. The kidney tissue cultures were either Blake or milk dilution bottle cultures with confluent growth.

Passage method. The "A" strain passage was begun using a 1-100 dilution of swine virus and passaging 0.1 ml of this into 9.9 ml of diluent. This procedure of a 1-100 dilution was carried out each time making the tenth passage a dilution of 1×10^{-23}. The "B" and "C" strains were initiated with a 1-10 dilution and thereafter carried out with the standard 1-100 dilution making the tenth passage a dilution of 1×10^{-22}. The tenth passages of both A and B strains were harvested from the same tissue culture batch while C was harvested from the succeeding culture.

The infectivity of VEV in tissue culture

Infectivity tests were carried out on normal and immune kidney and testicle tissue cultures. In the case of testicle produced virus titrations were also made in swine.

Virus. The virus used in each case represented an aliquoit of the tenth passage of either testicle or kidney material of strains "A", "B" and "C". Frozen samples held at -17 F were thawed out at room temperature, and unless otherwise noted, only a single freeze-thawing cycle was used.

Tissue culture titrations. Titrations were performed in tubes (150 mm x 15mm) of kidney and/or testicle tissue. Tubes of kidney tissue were usually 8 to 10 days of age, while tubes of testicle tissue ranged from 3 to 5 days at the time of inoculation. Dilutions of virus were made in the nutrient fluid such that each 2 ml quantity of nutrient per tube contained the desired virus dilution. Unless otherwise noted 10 tubes per dilution were routinely used. All tubes were incubated at 37 C and examined for evidence of cytopathogenic changes at 24, 48 and 72 hours after inoculation, at which time the titration was arbitrarily ended. TC_{50} endpoints were calculated by the method of Reed and Muench (1938).

Swine titrations. Titrations were performed in swine for manifold purposes:

to obtain some idea of the titer of the virus in swine and therefore direct evidence of the multiplication of the virus in tissue culture; to test the antigenic identity of the 3 strains being propagated in tissue culture; to prepare immune sera against the strains, and to obtain immune kidney and testicle tissue for tissue culture titrations.

A total of 9 swine test pig (TP) numbers 306, 7, 8, 10, 11, 12, 13, 14 and 15 were equally divided among pens 1, 2 and 3. All animals were then inoculated with 0.75 to 1.0 ml of *10AST10 intradermally into the snout and upper lip or intravenously. In pen #3 a 10^{-6} dilution was used, in pen #2 a 10^{-4} and in pen #1 a 10^{-2}.

Eighteen days later these same animals plus one additional control pig per pen (TP# 316,317,318) were challenged with 0.75 ml of a 1-10 dilution of known "A" type swine virus.

Following an interval of eighteen days these same animals plus an additional control animal per pen (TP #319, 320, 321) were inoculated as previously described with ◊10BST10. The animals in pen #3 were given a 10^{-6} dilution, those in pen #2 a 10^{-4} and in pen #1 a 10^{-2}. Certain of the animals in pen #1 (TP # 314, 318) which did not respond within 72 hours to the 10^{-2} dilution were reinoculated with 0.75 ml intradermally or intravenously with undiluted 10BST10. In addition one more animal, TP #322 was used as a control for the 10^{-0} dilution.

Eighteen days later all animals plus an additional control animal per pen (TP #323, 327, 325) were challenged with a 1-20 dilution of B type swine virus.

Again after an interval of 30 days these same animals plus one additional control per pen (TP #326, 327, 328) were given +10CST10 In pen #3 animals were inoculated with a 10^{-4} dilution, in pen #2 with a 10^{-2} and in pen #1 with undiluted virus.

Each animal was bled approximately 30 days after the last inoculation of C virus for at least 50 ml while certain ones donated 3 to 4 liters. Virus was harvested whenever possible.

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*10AST10 = 10th passage of "A" strain VEV on swine testicle.
◊10BST10 = 10th passage of "B" strain VEV on swine testicle.
+10CST10 = 10th passage of "C" strain VEV on swine testicle.

RESULTS

a) Tissue culture. The results of the first titration of strains "A", "B" and "C" in tissue culture are shown in tables 4 and 5. These data must be considered as being of a preliminary nature since time has as yet not permitted the retitration of the tenth passage nor subsequent titrations of other passages.

b) Swine. The results of the first titration of the tenth passage testicle virus in swine are shown in table 6.

DISCUSSION

The results of infectivity titers obtained in tissue culture indicate that there is a considerable difference in the ability of the various strains to multiply in tissue culture. Thus strain "A" gives a consistently higher titer than either "B" or "C" when cultivated on testicle and both "A" and "B" are higher than "C" when cultivated on kidney. In addition the titer is much higher when comparisons are made of virus cultivated in kidney versus testicle. These two observations lead to the assumptions that there may be actual strain differences between "A", "B" and "C" and that kidney is a better milieu in which to produce VEV than testicle. If one now examines the comparative infectivity titrations made on kidney versus testicle where the tissue culture origin of the virus is identical, one finds that the titers are of an equal magnitude. This latter points to similar susceptability of both types of tissue to VEV. The difference in titers seen with virus produced on testicle versus those on kidney may be a matter of differing cell populations; and not one of tissue susceptability. It has been noticed for example, that testicle tissue tends to grow in long horizontal strands whereas kidney grows in a confluent mat. This means that per unit area there are many more kidney cells than testicle and therefore more cells to produce virus. In part some of the differences between the titers of the different strains may also be explained on a similar basis. For example, both tenth passages of "A" and "B" strain kidney virus were produced in tissue from the same kidney lot (49) while "C" strain was produced from the next lot (50). It is possible that these two lots were not similar in cell population and this may account for the differences in titer. Similarly the tenth testicle passage of "A" strain was produced on one lot of testicle (13) while the "B" and "C" strains were produced on another lot (14). An examination of the titers of strains "A", "B" and "C" produced on testicle show strain "A" to have a higher titer than either strain "B" or "C".

It thus becomes apparent that where possible the same lots of tissue culture should be used to produce a given passage of the strains and these titrations compared before strain differences are used as an excuse for differences in titer.

As a part of the problem of the propagation of these viruses in tissue culture, the question arose as to what would happen if a kidney or testicle from an animal immune to VEV were used as a tissue culture source. To answer this the kidneys and testicles from an "A", "B" and "C" type immune animal were removed and used as a tissue culture source. The immune testicle failed to grow but the results on immune kidney indicated no significant differences between normal and immune kidney. The comparison will be repeated as immune tissues are made available.

Regardless of the variations noted above it is of primary importance to recognize that profitable titers are being obtained in tissue culture, and that within the limited scope so far attempted they are reproducible. Thus, for the first time a laboratory host is available for VEV.

The results of the titrations in swine of strains "A", "B" and "C" produced in testicle are unfortunately not as clear cut as those in tissue culture. There appears to be no question but that VEV propagated through 10 passages in tissue culture retained its viability and virulence for swine, and produced clinically typical vesicular exanthema. Strain "A" apparently possesses an infectivity titer of at least 10^{-2} but below 10^{-4}, since the swine inoculated with a dilution of 10^{-4} did not show clinical vesicular exanthema, and were not immune on subsequent challenge with swine virus 18 days later, while those inoculated with the 10^{-2} dilution responded with clinical vesicular exanthema and were immune to challenge . Thus, the titer of 10^{-2} should be compared to one of approximately 10^{-5} in tissue culture which indicates an approximate 3 log difference in infectivity between tissue culture and definitive host.

The results of the titration and challenge with the "B" strain were not as clear cut as those with "A" strain. The titer of the "B" virus was lower inasmuch as it appeared to lie between 10^{-0} and 10^{-2}. The tissue culture titer for this same virus appears to be about 10^{-3}, again representing a three log difference between tissue culture titer and swine titer. Challenge of these "B" type swine 18 days later with a 1:20 dilution of live swine virus resulted in all but one of the animals showing frank vesicular exanthema. The reason for this is not completely clear. The fact that those animals previously inoculated with dilutions of 10^{-0}, 10^{-4} and 10^{-2} should respond to a strong challenge virus is not surprising, since the tissue culture titer would indicate that very little virus was present even in a 10^{-2} dilution. However, those swine inoculated with the 10^{-0} dilution who actually responded with clinical vesicular exanthema were expected to be immune when challenged 18 days later with live swine virus. Why they were not is not clearly understood. Two possible explanations are advanced. The first is that the time span between infection with tissue culture virus and challenge with hog virus was too short for the development of clear-cut immunity and this coupled with a very high titer challenge virus simply overwhelmed whatever degree of immunity had been built up. The second is that some antigenic change had occurred with the tissue culture virus such that it was not capable of fully immunizing against the challenge virus. These speculations do little but indicate that further information is needed. The one fact obtained from this experiment was that the "B" virus was antigenically distinct from the "A".

The third series of inoculations concerned the "C" strain virus. The results were unexpected and are not understood. In the highest dilution used, 10^{-4}, 5 of 7 animals responded with frank vesicular exanthema. In the next dilution, 10^{-2} none of the animals responded and in the 0 dilution 2 of 8 had the diseases. Since the tissue culture titer of this virus ranged between 10^{-3} and 10^{-4} the swine virus titer might be considered to be 10^{-1} at the highest if the corallary with strains "A" and "B" of a 3 log difference between tissue culture and swine were to hold. Three possible explanations have occurred to the writer. The first is the obvious one, that the dilutions were mixed. This is believed to be out of the question since the 10^{-4} and 10^{-2} dilutions were inoculated on one day and the 10^{-0} was known to be inoculated on the following day. Thus it is believed that no possible technical error in the dilution series can account for the results.

A second possibility is that the virus was spread from pen #1 where the disease appeared 48 hours after inoculation to pen # 3 by man, where it did not appear until 7 days after the inoculation of the 10^{-4} dilution. That it did not appear in the pen #2 might be considered chance or the bare possibility that the amount of virus inoculated in pen #2 had been sufficient to partially immunize these animals against the challenge presented by the operators. This theory again is difficult to substantiate. It is the practice in this laboratory to begin work in the pen containing the greatest virus dilution first. Thus pen #3 containing the 10^{-4} dilution was examined first and pen #1 containing the 10^{-0} dilution last. Following each day's work all suits and gloves worn by operators are soaked in 2 per cent NaOH and subsequently thoroughly washed with hot water and hung to dry for the next day's work. It is hardly conceivable that virus could be carried from pen to pen. If carry over of this type were possible then certainly the virus should have been carried from pen #3 to pen #2. The third possibility is that these results represent a case of interference.

To increase the perplexity of the problem with the "C" strain five normal swine TP#329, 30, 31, 32 and 33 were given 0.75 ml of the virus either intradermally or intravenously and one animal was left as a contact exposure. The virus used was undiluted, first kidney passage of "C" strain. The results were inconclusive. One of the three animals inoculated via the intradermal route responded with clinical vesicular exanthema, the third showed no deviation from normal. The single animal inoculated via the intravenous route had clear cut vesicular exanthema as did the lone contact animal. These negative responses may represent occult cases such as Mott, Patterson, Songer and Hopkins (1953) have described.

Thus the results in swine with three strains of virus produced on testicle show that: (1) the three strains of the virus were passaged through 10 passages and (2) the "A", "B" and "C" strains derived from these tenth passages are apparently antigenic entities.

SUMMARY

The aggregate of the results obtained to date clearly indicate that tissue culture is a method of producing infective virus and one permitting the subsequent titration of that virus in a host other than swine. It is believed that this system will be adequate to produce the required prophylactic agents and opens an entirely new field for the characterization of the fundamental properties of the vesicular viruses.

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TABLE 1

Incidence of vesicular exanthema in California for the period 1932 to 1952, showing number and type of infected premises*

Year	Number of outbreaks according to the types of premises			Number swine involved	Total swine in state	Per cent total swine infected
	Garbage feeding	Grain feeding	Slaughter house			
1932	5	0	0	18,747	672,000	3
1933	3	0	0	5,533	706,000	0.7
1934	31	0	0	95,917	660,000	14.4
1935	4	0	0	10,100	530,000	2.0
1936	14	0	0	13,625	610,000	3.1
1937	0	0	0	0	732,000	0
1938	0	0	0	0	820,000	0
1939	15	0	0	32,000	763,000	0.4
1940	161	7	1	277,250	885,000	31.3
1941	155	15	0	160,104	876,000	18.0
1942	15	0	0	84,300	894,000	0.9
1943	122	3	14	288,355	1,019,000	28.0
1944	154	7	10	429,876	1,060,000	41.5
1945	58	0	2	127,620	763,000	16.7
1946	52	0	1	108,732	717,000	15.2
1947	129	10	4	212,535	664,000	32.0
1948	25	0	0	84,566	641,000	13.0
1949	101	0	4	199,875	671,000	29.8
1950	169	6	9	272,222	687,000	39.7
1951	53	1	4	82,442	653,000	12.4
1952	105	4	107	224,976	610,000	37.0
Totals	1,371	53	156	2,739,275	12,418,000	----

* There are approximately 20,000 premises on which hogs are raised in California. Four hundred of these are garbage feeding and have a hog population of about 230,000/annum, the remaining 19,600 are grain feeding. Modified from Duckworth (1953).

TABLE 2

Clinical response of the three vesicular viruses in the important hosts and by various routes of inoculation *1

Test species	Route of inoculation	No. animals needed	Response expected if unknown virus is:		
			VE	VS	F and M
Swine	Intradermal snout, lips, plus scarified snout.	2	+	+	+
	Intravenous	1	+	+	+
Horse	Intramuscular	1	$\bar{+}$	-	-
	Intralingual	1	$\pm$	+	-
Cow	Intradermal tongue, gum and lips	1	-	$\bar{+}$	+
	Intramuscular	1	-	-	+
Guinea Pig	Intradermal volar surface of the plantar pads	2	-	+	+

+ Produces typical disease process - No clinical reaction $\bar{+}$ Disease process rarely occurs

$\pm$ Usually very mild evidence of vesicular disease approximately 50 per cent of the time

*1 Modified from Madin and Traum (1953).

TABLE 3

Status of state legislation as of August 1953

requiring the cooking of garbage before being fed to hogs +

States with legislation	States with regulations	Legislation defeated	No action
Alabama	Colorado	California ◊	Arkansas
Arizona	Kentucky	New Jersey	Delaware
Connecticut	Maryland	New Mexico *	Vermont
Florida	Mississippi	North Dakota	
Georgia	Nevada	Rhode Island	
Idaho	Virginia		
Illinois			
Indiana			
Iowa			
Kansas			
Louisiana			
Maine			
Massachusetts			
Michigan			
Minnesota			
Missouri			
Montana			
Nebraska			
New Hampshire			
New York			
North Carolina			
Ohio			
Oklahoma			
Pennsylvania			
South Carolina			
South Dakota			
Tennessee			
Texas			
Utah			
Washington			
West Virginia			
Wisconsin			
Wyoming			

+ Source — Modified from Veterinary Public Health Communicable Disease Center
◊ California will not accept hogs for slaughter fed on raw garbage since 1954
* New Mexico recommends no garbage feeding

TABLE 4

Infectivity titers of the tenth passage of VEV produced in testicle tissue cultures with cross titrations in normal and immune kidney and testicles

Virus type	Cultivated on	Dilution inoculated	Results of infectivity titer on					
			Normal kidney	TC_{50}	Immune kidney	TC_{50}	Testicle	TC_{50}
A	testicle	10-1	*ND	5.60	ND	4.60	10/10	> 5.00
		-2	ND		5/5		10/10	
		-3	ND		5/5		10/10	
		-4	10/10		3/5		10/10	
		-5	6/10		0/5		8/10	
		-6	1/10		0/5			
		-7	0/10					
		-8	0/10					
B	testicle	10-1	ND	2.67	10/10	2.50	10/10	3.50
		-2	10/10		8/10		10/10	
		-3	4/10		1/10		10/10	
		-4	0/10		0/10		2/10	
		-5	0/10		0/10		0/10	
		-6	0/10					
C	testicle	10-1	10/10	3.17	4/5	2.62	10/10	4.4
		-2	10/10		1/5		10/10	
		-3	6/10		0/5		8/10	
		-4	0/10		0/5		1/10	
		-5	0/10		0/5			
		-6	0/10					
		-7	0/10					

* ND = Not done

TABLE 5

Infectivity titers of the tenth passage of VEV produced in kidney tissue cultures with cross titrations in normal and immune kidney and testicle

Virus type	Cultivated on	Dilution inoculated	Results of infectivity titer on					
			Normal kidney	TC_{50}	Immune kidney	TC_{50}	Testicle	TC_{50}
A	kidney	10-1	*ND		ND			
		-2	ND		ND			
		-3	ND		ND			
		-4	10/10	7.0	7/7	6.57	10/10	7.4
		-5	10/10		7/7		10/10	
		-6	10/10		7/7		10/10	
		-7	5/10		0/7		7/10	
		-8	0/10		1/7		1/10	
B	kidney	10-1	ND		ND			
		-2	ND		ND			
		-3	ND		ND			
		-4	10/10	6.62	7/7	6.51	10/10	7.5
		-5	10/10		7/7		10/10	
		-6	10/10		6/7		10/10	
		-7	2/10		1/7		8/10	
		-8	0/10		0/7		2/10	
C	kidney	10-1	ND		ND			
		-2	ND		ND			
		-3	ND		ND			
		-4	10/10	5.0	6/7	5.40	10/10	4.8
		-5	4/10		5/7		3/10	
		-6	2/10		2/7		1/10	
		-7	0/10		0/7		0/10	
		-8	0/10		0/7		0/10	

*ND = Not done

TABLE 6

TEST PIG NUMBER	INFECTIVITY OF 3 SEROTYPES OF THE TENTH TESTICLE PASSAGE VIRUS & RESULTS OF IMMUNITY CHALLENGE: 0.5 ML. TO 1.0 ML. INOCULATIONS														
	A						B						C		
	TESTICLE VIRUS			CHALLENGE SWINE VIRUS			TESTICLE VIRUS			CHALLENGE SWINE VIRUS			TESTICLE VIRUS		
	ROUTE	DILUTION USED	RESULTS	ROUTE	DILUTION USED	RESULTS	ROUTE	DILUTION USED	RESULTS	ROUTE	DILUTION USED	RESULTS	ROUTE	DILUTION USED	RESULTS
306	ID	10^{-3}	N	ID	10^{-1}	S & L − − − +	ID	10^{-6}	N	CONTACT		S & L − − − +	ID	10^{-6}	N − + − −
307	ID		N	ID		S & L − − − +	ID		N	CONTACT		NO P − + − −	ID		N − + − −
308	IV		N	ID		S & L + − − +	IV		N	ID	2×10^{-1}	P + + + +	ID		N − + − −
316				ID		S & L + + + +	ID		N	CONTACT		P + + + +	ID		N
319							ID		N	ID	2×10^{-2}	P + + + +	ID		N + − − −
323										ID		P + + + +	ID		N
326													ID		P − + + +
310	IV	10^{-4}	N	ID	10^{-1}	P − − − −	IV	10^{-4}	N	CONTACT	2×10^{-1}	P − − + +	ID	10^{-2}	N
311	ID		N	ID		P − − + +	ID		N	CONTACT		P + + + +	ID		N
312	ID		N	ID		P − − − −	ID		N	ID		P + + + +	ID		N
317				ID		P + + + +	ID		N	CONTACT		P − − + −	ID		N
320							ID		N	ID		P − − − −	ID		N
324										ID		P + + + +	ID		N
327													ID		N
313	ID	10^{-2}	N	ID	10^{-1}	N	ID	10^{-2}	N	CONTACT		P + + − −	CONTACT		N
314	IV		NO P + + − −	ID		N	IV	10^{-2}_{-0}	N P − − − −	CONTACT		NO P + − − −	ID		NO P + − − −
315	ID		P − + − −	ID		N	ID	10^{-2}	N	ID		P − + − −	ID		N
318				ID		P − + + −	ID	10^{-2}_{-0}	N P − − − −	CONTACT		N	ID		N
321							ID	10^{-2}	N	ID	2×10^{-1}	P + + + +	ID	10^{-0}	N
322							ID	10^{-0}	P + + + +	ID	2×10^{-1}	P − + + +	ID		N
325										ID	2×10^{-1}	P − − + +	ID		P + + − −
328													ID		N

N = NEGATIVE
P = PRIMARIES
NO P = NO PRIMARIES
S & L = SNOUT AND LIP LESIONS
+ AND − : NEGATIVE OR POSITIVE VE ON LEFT FRONT, RT. FRONT, LEFT REAR, RT. REAR FOOT

Figure 1. Outbreaks of vesicular exanthema in California 1932 to 1952.

(by counties)

(NP57-1191)

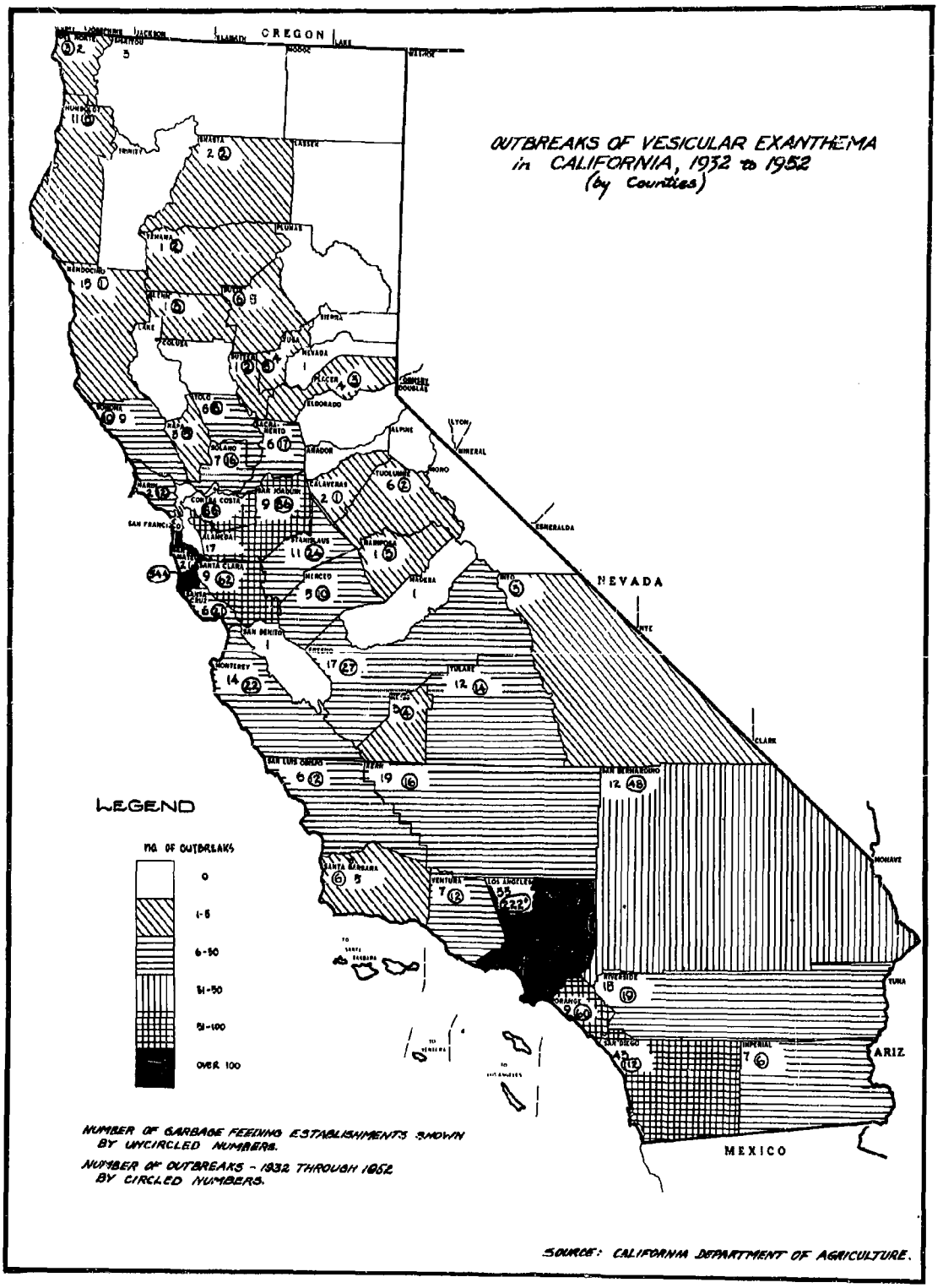
OUTBREAKS OF VESICULAR EXANTHEMA
in CALIFORNIA, 1932 to 1952
(by Counties)
OREGON
NEVADA
ARIZ
MEXICO
LEGEND
NO. OF OUTBREAKS
0
1-5
6-50
51-50
51-100
OVER 100
NUMBER OF GARBAGE FEEDING ESTABLISHMENTS SHOWN BY UNCIRCLED NUMBERS.
NUMBER OF OUTBREAKS - 1932 THROUGH 1952 BY CIRCLED NUMBERS.
SOURCE: CALIFORNIA DEPARTMENT OF AGRICULTURE.

Figure 2. Number of vesicular exanthema infected-exposed swine during national outbreak.

(NP57-1189)

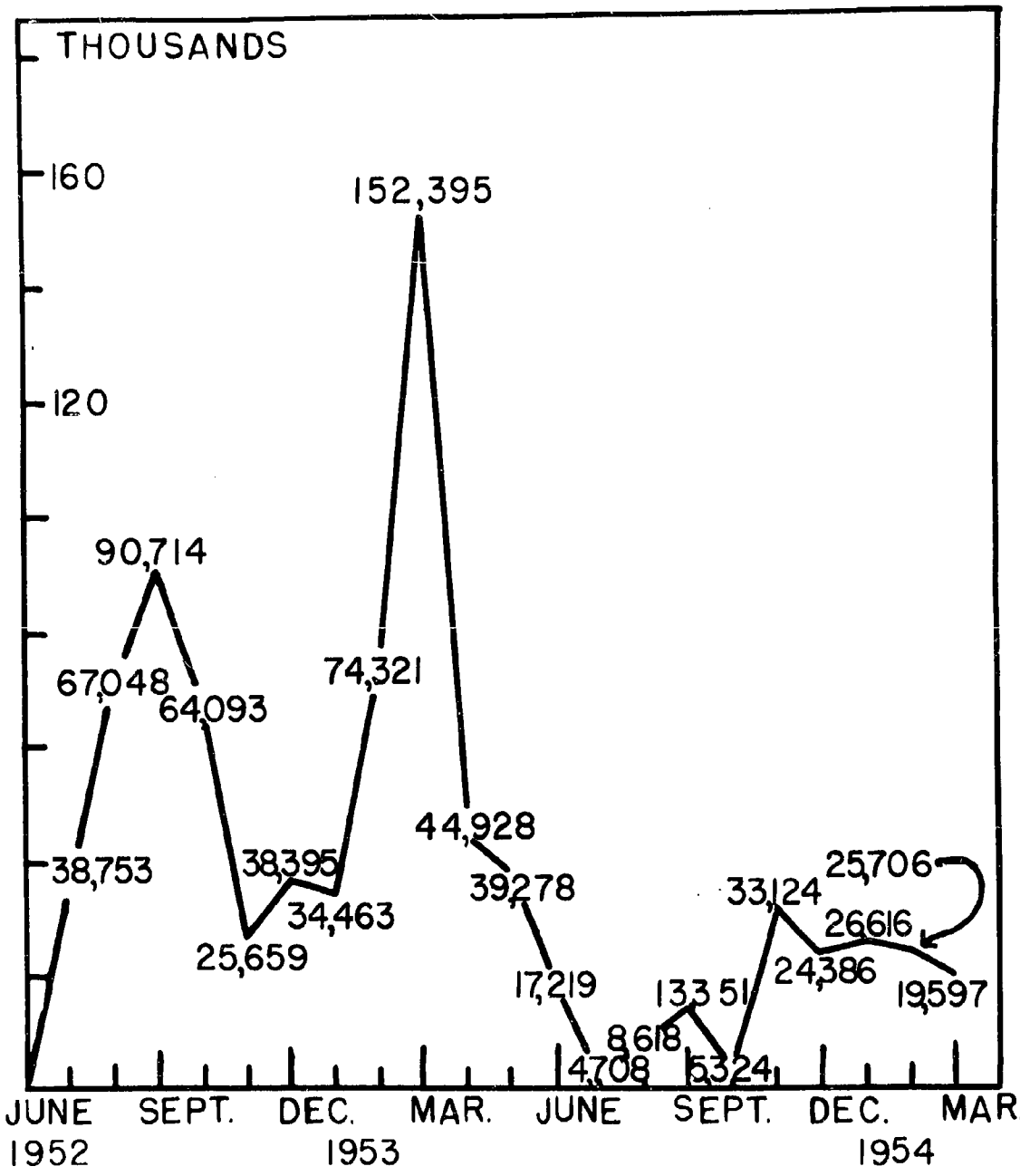
THOUSANDS
160
120
152,395
90,714
67,048
64,093
74,321
44,928
38,753
38,395
39,278
34,463
25,706
33,124
26,616
25,659
24,386
17,219
19,597
13,351
8,618
4,708
5,324
JUNE
SEPT.
DEC.
MAR.
JUNE
SEPT.
DEC.
MAR.
1952
1953
1954

Figure 3. Characteristic temperature curve following intradermal inoculation of swine with vesicular exanthema virus.

(NP57-1190)

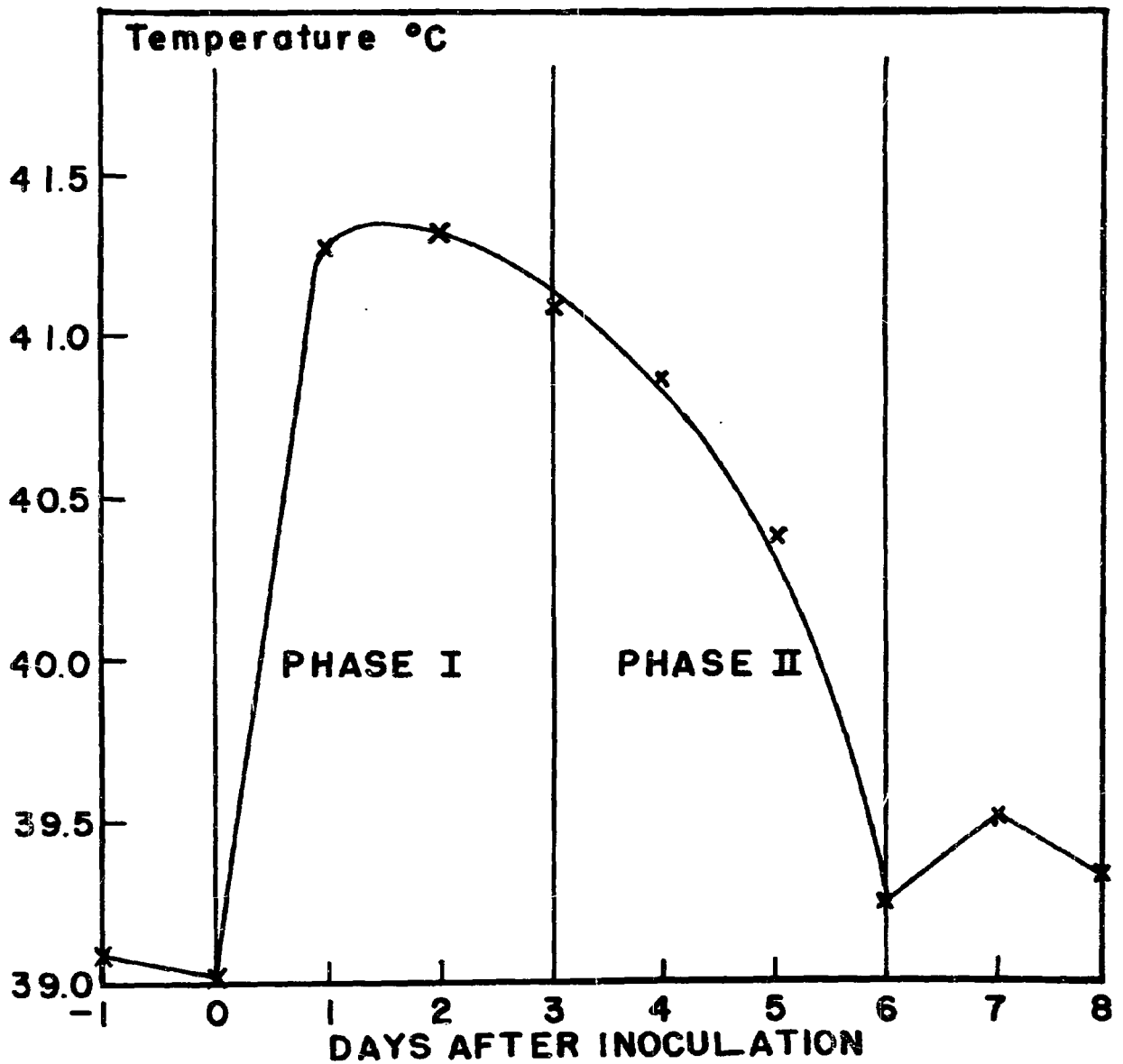
Temperature °C
41.5
41.0
40.5
40.0
39.5
39.0
PHASE I
PHASE II
-1
0
1
2
3
4
5
6
7
8
DAYS AFTER INOCULATION

Figure 4. Normal swine snout expithelium (Giemsa, 100 x).

(NP57-1184)

Figure 5. Vesicular exanthema infected swine snout epithelium (Giemsa, 100 x). (NF57-1185)

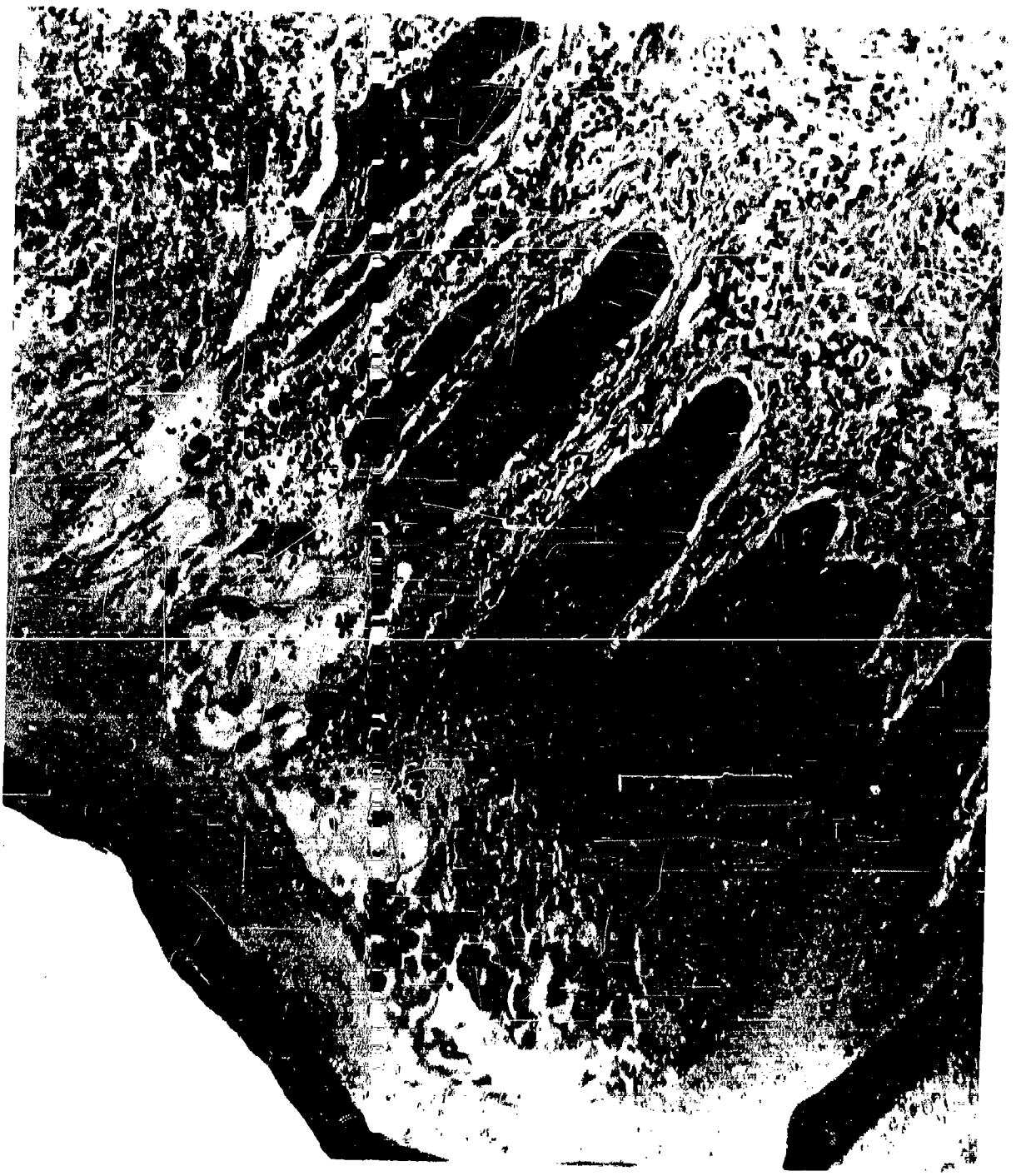

Figure 6. Normal swine snout epithelium (Giemsa, 1000 x).
(NP57-1187)

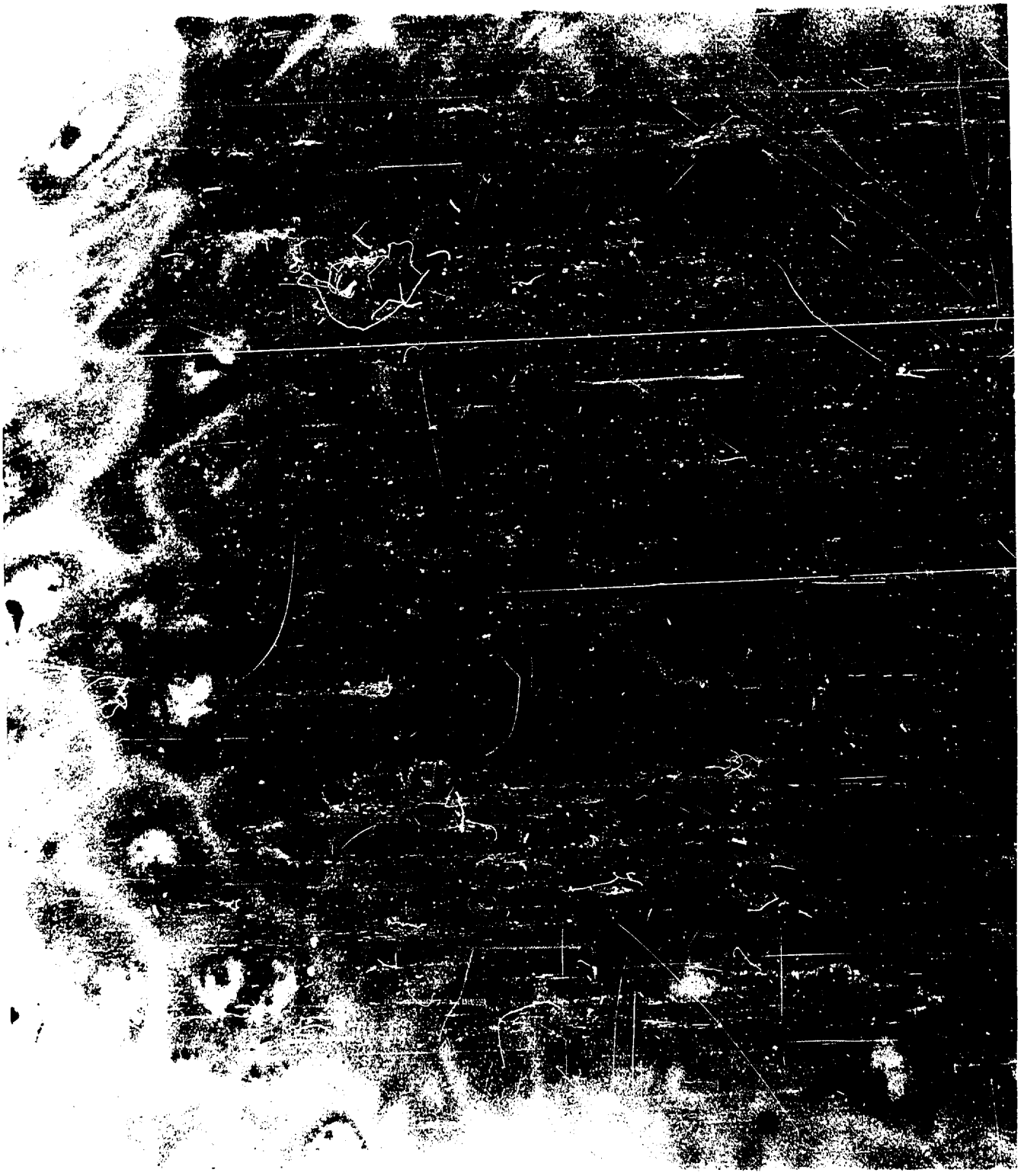

Figure 7. Vesicular exanthema infected swine snout epithelium (Giemsa, 1000 x).

(MF57-1186)

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